

## **Team Members:**

### **SVCE\_CHENNAI 2016**

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## **Attributions (Lab Troubleshooting)**

- Tec-Chihuahua
- IISC\_Banglore
- UPO Sevilla
- Warwick
- Oxford
- Edinburgh UG
- Team Technion
- Team Kent

## **PART ONE- LABORATORY**

### **CLONING TROUBLESHOOTING MADE SIMPLE:**

#### **PREAMBLE:**

Once Team SVCE Chennai started the lab work which involved a major work of cloning, multidimensional problems were experienced. These problems were due to various reasons, which were identified and resolved. We went through a tedious yet fun filled process in troubleshooting and finding optimum solutions.

Here, we would like to share few troubleshooting methods in cloning.

This guide has been prepared for the new teams in (2017 and so on) and takes the reference of various website information in a nut shell.

The iGEM teams were approached for the lab troubleshooting guide and hereby we are happy to say that the teams (team members and the team advisors) mentioned below helped us in framing this guide, with their wet lab experience.

The teams are as follows:

- Tec-Chihuahua
- IISC\_Bangalore
- UPO Sevilla

- Warwick
- Oxford
- Edinburgh UG
- Team Technion
- Team Kent

Have fun looking into our troubleshooting guide!!

## **TROUBLESHOOTING MADE SIMPLE!**

### **TRANSFORMATION:**

Transformation is done on an average of 6-8 times per week and it is felt that 75% of the time, expected efficiency isn't reached.

Predominant problems faced by the teams and the solutions for those are as follows:

- Defective commercial competent cells
- Old/damaged DNA sample
- Lack of oxygen for cells( inappropriate incubation conditions)
- Colonies obtained in the control plate

- Very low transformation efficiency
- Colonies do not grow always and when they do, they do not have the right inserts( sometimes).There is a fluctuation in the results.
- False positive results in blue white screening

**Reasons-** cells not viable/ competent cells inefficient, antibiotic problem(concentration), protocol problem (heat shock timing in particular), problems related to the insert( its toxicity, size, plant or mammalian source etc, and incomplete insert due to inefficient ligation).

## **Solution:**

- Remaking of competent cells/purchasing commercial competent cells.
- Antibiotic concentration to be optimized. (MIC of the antibiotic is needed to be used. This can be assessed by broth dilution test/agar dilution test).

Recommended working concentrations of:

- Ampicillin- 100ug/ml
- Kanamycin-50-75ug/ml
- Chloramphenicol-25-50ug/ml

(as per optimization)

- For toxic DNA inserts, lower incubation temperature (25-30°C) will be useful. (To prevent the toxicity by recombinant DNA, the recombinant DNA fragment should be cloned in the site flanked by some repressor such as the with multiple operators . These vectors will bind to repressors to make the DNA fragment inaccessible to the cellular factors and therefore eliminate the toxicity.)Also, competent cells which show higher efficiency due to strong transcriptional factors could be used.
- Heat shock 30 seconds (42°C exactly) -2minutes. (Depending on your sample and laboratory conditions).
- Vortexing should not be done once the plasmid is added to the cell culture. Gentle mixing is appreciable.
- Placing the mixture before heat shock, on ice for 30minutes is highly crucial. Every 5 minutes lessened will reduce the efficiency in significant amounts.
- For handling large constructs, high efficiency competent cells are to be used. (eg: XL 10-Gold Ultra competent cells possess the high transformation phenotype (The), which gives these cells an average transformation efficiency of  $\geq 5 \times 10^9$  transformants/ $\mu\text{g}$  of supercoiled DNA and makes them ideal for the transformation of large plasmids and ligated DNA).

- If the construct is susceptible to recombination, cells with Rec A strain with high stability and efficiency are to be chosen.

(generally retroviral genes contain clones with inverted repeats or other sequences prone to recombination).

- If the source of DNA is plant/mammal, then the sample will contain methylated cytosines(DNA methylation) and thereby ,cells lacking McrA, McrBC and Mrr systems that degrade this, have to be used during transformation.
- Optimal time of ligation and using 1-4ul of ligated mixture for transformation increases efficiency.( extended ligation time may lower the efficiency)
- Molar ratio of vector insert can be varied from 1:1 to 1:10 ( 1:2 ratio worked for our team).
- Ethanol precipitation and clean drying of pellet before resuspending is essential to avoid contaminants ( eg isopropanol,ethanol,phenol etc).
- Generally the five simple techniques for verifying the transformed colonies are:

- Blue white screening
  - Positive selection vector
  - Colony PCR
  - Sequencing
  - Diagnostic restriction digest
- 
- Troubleshooting false positive results in blue white screening:
    - Though blue white screening is effective in verifying recombinants, only a single attempt is not effective. Secondary screening is required. Two methods can be used to investigate the accuracy of secondary blue-white screening for target colonies on lysogeny broth (LB)-ampicillin agar plates. The first method consists of covering the target colonies grown on LB-ampicillin plate medium with a sterilized filter paper soaked in a solution of 60  $\mu$ L 20 mg/mL X-gal and 8  $\mu$ L 20% IPTG. The second method is that blue and white colonies could be randomly selected from the blue-white screening plate medium and then re-streaked onto the blue-white screening medium. The colonies are then incubated at 37°C for 12 h. The results showed that some of the white colonies treated by the two methods show results similar to the colonies grown on the blue-white screening medium. These results indicate that



the target colonies grown on blue-white screening medium can still be used to carry out a secondary blue-white screening. Thus, false positives can be eliminated directly based on the color of the target colonies. ([Zhang YS<sup>1</sup>](#),2016)

Further issues in transformation would be due to improper restriction digestion, ligation and PCR methods. The troubleshoot for these will be discussed below.

## **POLYMERISED CHAIN REACTION:**

### **Main problems experienced by the teams:**

- Appearance of bands of inappropriate size
- No appearance of band
- Multiple bands appear

**Reasons-** Damaged DNA/toxic DNA, inefficient polymerase, buffer issue, incorrect annealing temperature, extension time, insufficient number of cycles, contamination of the sample, nonspecific amplification, contamination in the equipments used/handling errors.

## **Solution:**

- Choosing a high fidelity polymerase will make the process easy. ( eg: phusion polymerase).
- The extension time and the number of cycles for the sample need to be optimized. They vary with the size of the sample.
- Sometimes the damage of DNA may be caused due to increased time spent in analyzing it under UV. This time can be reduced.( rare case)
- If the DNA sample is found to be toxic, it can be amplified by cloning into a non-expression vector.
- The annealing temperatures must be in accordance with the primer  $T_m$  values. Annealing temperature gradient could be tested for optimization.
- The sequence of the primer has to be thoroughly checked.( the oligos must be exactly complementary to the target sequence).
- The DNA sample can be purified prior to PCR by alcohol precipitation.

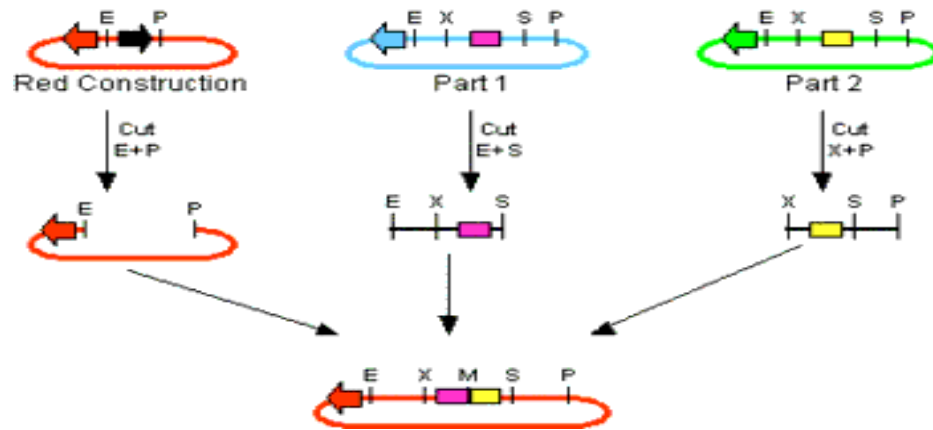
- $Mg^{++}$  and KCl concentrations are very important in maintaining the efficiency of PCR. Too low  $Mg^{++}$  will yield low product, whereas too high  $Mg^{++}$  will yield nonspecific products. The  $MgCl_2$  concentration should normally be between 1mM and 4mM. Changing the dNTP, KCl and any other concentration in the reaction mixture would require a change in the concentration of  $MgCl_2$ .
- The size of the product is inversely proportional to the salt concentration. Normal KCl concentration-50mM. For a size range of 100-1000bp, KCl concentration- 70-100mM.( recommended)
- Nonspecific amplification could be reduced by increasing KCl concentration and number of reaction cycles.
- The usual dNTP concentration is between 40 $\mu$ M and 200 $\mu$ M of EACH of the four dNTPs.
- For GC rich templates, usage of GC buffer instead of High fidelity buffer is recommended. If the DNA has to be free from detergent, Detergent free buffers could be used.
- Enzyme amount – generally 0.5-2 units per 50ul of reaction depending on the size of the amplicon.

- Template concentration- 1pg-10ng/50ul ( low complexity DNA like lambda) and 50-250ng/50ul (for high complexity DNA) . It should not increase 10%concentration of the final mixture to be obtained.
- Extension (72°C) time varies from 15 seconds to 40 seconds depending on the complexity of DNA.
- Addition of DMSO with different polymerases(phusion, taq,platinum taq,Q5 polymerases) and their respective buffers may increase PCR efficiency.( by facilitating proper annealing)

## **RESTRICTION AND LIGATION:**

**Regarding restriction, the restriction sites available in PSB1C3 plasmid provided by iGEM are, E,X,S and P. The appropriate restriction enzymes are to be used.**

## **3A ASSEMBLY**



**Problems faced** -Though restriction hasn't been a major problem for many teams, ligation has been. Improper ligation, presence of sample of inappropriate size ,no sample seen in the gel are few issues related to restriction-ligation part of cloning.

### Solution:

- If double digestion seems to be inefficient, the main parameters to be checked are the buffer concentrations, and compatibility of buffers. Generally the buffer suitable for both the enzymes used is preferable.( Tango buffer suits almost all the three enzymes except ECOR1)
- Sequential digestion could be carried out but the elution of the sample after the first digestion should be taken care of, because sample loss is quite often to occur.

- The restricted vector has to be treated with alkaline phosphatase for the efficiency of ligation. (After digesting the vector (and blunting, if appropriate), treat with phosphatase: 1 hr at 37°C for 5' overhangs, or 1 hr at 50°C if the DNA has any blunt ends or 3' overhangs.)
- Purification of phosphatase is necessary( with spin column).
- Make sure that no droplet escapes the enzyme treatment.
- Ligation time needs to be optimized based on the type of insert, its sticky ends and blunt ends.( ranges from 10minutes to 4 hours and overnight ligation).

Few common conditions opted by the teams are as follows:

- 1 hour ligation followed by water bath(80°C) for twenty minutes
- 1:1 ligation insert vector ratio, 37°C, 1 hour
- NEB ligation protocol( T4 DNA ligase), 14°C for atleast 16 hours
- 13ul insert, 4ul plasmid, 2ul t4 ligase buffer and 1ul t4 ligase and then ligate overnight at 16 degrees.
- 10 minute quick ligation protocol.

An in depth assessment of these factors and related ones will help in the optimization of cloning as a whole process

## **PART TWO : HUMAN PRACTICES**

### **PREAMBLE:**

After team SVCE\_Chennai zeroed in on working with antimicrobial peptides to address the issue of milk spoilage, we realized that we had to consider its impact on the society. We paralleled our lab and human practices work and made sure we executed them in the proper way. Various hurdles and obstacles were faced by the team and every step led to progressive learning. Based on our learning, we would like to share our ideas on how every compartment of iGEM work can be approached and performed productively.

We reviewed the works of many iGEM teams and also the official iGEM guide page and, on the whole, we share our inference in this page.

### **OUR JOURNEY IN HUMAN PRACTICES WORK:**

Once we started with our iGEM project, we pooled in various ideas for the outreach work. Creating awareness about the issue, we felt, is the most important of everything. Before the awareness program, we thought we had to gather people's opinion on the intensity of the issue and thereby felt the importance of statistical analysis. Also, the statistical data had to be effective. For this, we gained opinion from different sections of the society through surveys (urban, rural, student

community). We also gained opinions from people in different parts of the world, through surveys.

The next part of our work, after analyzing the issue, we felt, is to spread awareness on various topics, especially , the milk spoilage issue, importance on GMOs, importance of Synthetic Biology, and many more. We created awareness by different methodologies to the public as well as the student community. (Urban education and engagement, rural education and engagement, student education via workshops, symposiums, meetings, camps etc- visit our HP page for more details.).

This was followed by our visit to different stakeholders of the society so that we could have their feedback and extract the essence out of it. Also, we worked for the commercialization of our product by consulting milk industries and government officials. By meeting them, we got many ideas on how to improve our product and how to implement it in the present scenario.

Human practices is not only about spreading awareness and gaining opinions seriously, but it is also about having fun! We created game apps and videos and shared it via social networking sites to people of all ages, so that learning could happen in a fun filled way.

With this base, and with reference to the outreach work done by the iGEM teams, we hereby pool in various HP ideas for the benefit of new iGEM teams:

- iGEM Human practices work can be done effectively in a period of six months. If the team hasn't scrutinized any idea, the team could form a **public hub** (both online and offline) in which people from different communities can discuss a wide range of issues or multifarious parts of a particular issue.



This will help the team to narrow down the subject of interest and find an optimum solution.

(Note: If the project is an entertainment based one, with the aid of synthetic biology, even then, awareness can be created to the society based on the concepts of your project)

- Once the idea pops out , gearing up with HP work and programs is the ultimate aim for the team. The main objectives could be listed , followed by the work split up. Once the work is split up in various sub teams, deciding the deadline for every work is essential. A clean, detailed discussion with negotiations in the team will give way for constructive ideas, leading to productive results in the end. Every person in the team is unique in his/her own way, and has special talents and qualities. Tasks could be delegated to the ones with the appropriate skills, so that the best out of every person is brought out, for the benefit of the team.

This way, an agenda for the team work can be detailed and executed progressively, in a stepwise manner.

- A thorough **check of the iGEM guidelines and medal criteria** is essential to perform accordingly.

- **A meticulous and methodical study** of the project is necessary. The diversified issues that the product may encounter in the society have to be analyzed. The issues include:
  - **Ethical issues** – There is a need to check if the project is free of ethical issues( bio ethics and other related issues).They fall into three sections namely , the ones based on methods used, application issues of the project and the distribution of the product(concept) within various sections of society. ([Deplazes et al., 2010](#))
  - **Social issues**- Considering the impact of the project in society is essential. The utility-economic impact gradient is one the important criteria to be assessed especially if the team intends to compete for the entrepreneurship award.
  - **Legal issues**- These go hand in hand with the rules and regulations of the respective country's regulatory body and the team has to check if their product satisfies all the requirements and conditions.
  - **Biosafety and biosecurity issues**- The issues connected with the laboratory methods used as well as the matters related to the safety issues during the product release need to be considered.

Clearing all these issues will make the project a safe and sustainable one.

- Once the fields mentioned above are assessed in detail, the team can spread the word that their project is a safe and sustainable one, to everyone around through various methods.
- Not only about your project , but also general **“spread the word** “concept for recognizing many concepts related to synthetic biology can be done.

(These include Biosafety,laboratory safety, biohackery-do it yourself biology,biodefense, bioeconomy,safety regarding biobricks and what not!)

- A **festival or social event** could be organized (for the public) to propagate your idea along with some fun element. These may include:
  - Public rally
  - Vigil
  - Debating platforms

- Party etc.
- If your team intends to produce a marketable product (nonspecific) which has a wide range of novel applications, interact with the urban/rural public/student community on what ways they would use your product for. This would **inculcate business ideas** in them (especially the young minds), and who knew, you might be creating new entrepreneurs!
- **Expertise from various related fields** could be contacted and discussions regarding your project could be conducted. Not only your project, but also general issues related to synthetic biology can be discussed upon. (Gaining opinions and views from experts is highly important to refine your project idea)
- **Create a platform (online/offline)** where these experts could share their knowledge with others ( public, your teammates, other experts), so that the idea is more refined and the many are benefited out of it.
- Checking the **application and techno moral scenarios** of your project and targeting these while organizing public events will help to

fine tune the project. This will help in getting your project funded by institutions like Synergene, Syngenta etc.

- To extend the idea of these scenarios, **stakeholders from different segments** (scientists, policymakers, doctors, journalists, professor's ministries, professional advisors etc) could be approached. Again, this will help you in commercialization of your product.

(Remember that every criticism will aid in the betterment of your project).

- **Communication with people who have already worked on stuffs related to your idea** is essential. Eg: If you intend to produce a biomarker for lead toxicity, try contacting experts who have already worked in it, and refine your idea accordingly.
- Not only can you organize your own events, but also **participate in events organized by other popular bodies**( say college symposium etc) and publicize .

- **Workshops** could be conducted on a broad spectrum basis.(especially for students of various grades).Nothing outstands hands on sessions!!
- **Be connected with the other iGEM teams** through social networkinhg sites like facebook,twitter etc and create a community for all the iGEM teams where ideas could be pooled in and criticisms encouraged for the benefit of all the participants.
- Keep everyone (in iGEM and the outside world) updated of what your team is up to. This includes **hosting videos and related stuffs in you tube channels.**
- Be connected with various **small scale and large scale industries** related to your field.
- **Booklets and magazines** for the student world (which contain both your project topics as well as other stuffs) could be made.
- **Newspapers, TV shows** and other media could be used to propagate your idea.

- **Presentations, games, fun events** could be organized in **schools and colleges**. Research ideas could be seeded in young minds which would encourage them to pursue their future in the ocean of research and development.

**Safety committees** all around need to be consulted which would help the team to avoid safety issues in future. This would also help the team to take a step in patenting in future.

### **PART THREE: COLLABORATION GUIDE:**

Collaboration is a way of building mutual understanding and assistance among the various iGEM teams. Every year in the Giant Jamboree, special awards and prizes are given to teams which are best in collaboration. And the best part is that it is one among the silver medal criteria. So here we are giving you a set of ideas and suggestions your team can do to be one among the award winners. You can collaborate with other iGEM teams by the following ways:

- **Collaboration with the other iGEM teams through mutual exchange of surveys:**

Survey forms and questionnaires regarding the concept of your project or even regarding synthetic biology could be created and sent to other teams, requesting them to circulate it across their region and the responses could be compiled. Thus a mutual exchange of surveys could be done this way. For instance, We actively collaborated with many teams and exchanged our surveys based on both of our projects and also commuted a survey based on troubleshooting lab techniques which was filled by the following teams:

- i. **Team Oxford**
- ii. **Team Edinburgh**
- iii. **Team Warwick**
- iv. **Team UPO Sevilla**
- v. **Team Tec-Chihuaua**
- vi. **Team Kent**
- vii. **Team IISC Bangalore**
- viii. **Team Technion**

- **Skype calls and meet-ups:**

You can connect with other iGEMers through Skype calls and meetings to have discussions and presentations on both of your projects. This will help your team to have a pre-iGEM experience of presenting your project before different teams and will induce your confidence. Also the discussions carried out during the meet facilitates the improvement of your project.



- **Interchange bio-brick parts:**

You can mutually interchange your bio-brick parts with other iGEM teams and help characterizing it by observing it's dynamics at a different circumstance and also by modifying it and making it more efficient. Another great way would be sending the samples from your region to the other team to help them analyze their construct's efficiency in it (e.g. Soil samples). These will hold good for the benefit of each other.

- **Assist in lab troubleshooting:**

- You may conjointly assist each other in troubleshooting the problems you face in lab viz., with cloning, PCR techniques and so on. Framing a standard protocol for a particular technique done in the lab (e.g. transformation) and sending it to other teams will also be encouraged to a greater extent.

- **Help in wiki-styling:**

- Mutual help may be done in designing and personalizing your wikis and also help in collecting more information for it.

- **Team Meet-ups:**

- Meet-ups could be organized with other teams from your country in person or even with teams abroad through Skype to present your project and also to have mutual assistance in your project.
  
- **Provide project-related guidance:**
  - Guidance may be given mutually to narrow down the idea for your project and also to frame the teams. Once you have arrived at your project, you can aid the other teams by providing literature back-up regarding their work, by acquiring the perspectives of people about their idea and also by offering the contacts of scholars and scientists with whom they can talk for the enhancement of their work. A workshop or lecture session can be conducted wherein you can demonstrate the lab techniques to other teams.
  
- **Create databases:**
  - Generation of a database exclusively on a particular domain (e.g. biosensor) which includes data about the previous iGEM projects related to that domain can be done by joining hands with another team. This will greatly influence the future and as well as the present iGEMers to be instantly availed of the information they require.

- **Form a community:**

- An iGEM community could be built with the help of other teams where you can enroll the information about each and every iGEM team along with their projects described in the simplest way. This will give way to couch -surfing and help the iGEM teams to be connected and as well as make way for them to collaborate with other teams who are doing similar kind of projects. Thus your community will serve also serve as an information bank.

- **Spread the word together:**

- Combining with other teams and creating awareness about your projects among the public by conducting workshops, visiting supermarkets, malls, etc will surely enhance your work to a greater extent.
- Make sure the collaboration is done with both the teams in your country as well as other places. This will enhance your collaboration efficiency.
- Surf through the iGEM page to check out which teams are working on the same concept as yours. If found, collaborate with them to enhance the quality of both the projects.

- Assisting new teams to take significant steps in the competition is a good way of collaboration.
- Also, to improve your project, collaboration could be done with non-iGEM organization's (say industries).
- You could help the other team by improving the function of their device (product) by targeted methods in your laboratory.
- You could assist another team in software and hardware handling (eg mathematical modeling).
- Remember! Collaboration includes both providing help as well as gaining help! So you could gain support in all these ways and other targeted ways for your project!!

Happy collaborating!!

## **PART FOUR: FUNDING GUIDE:**

”Money makes many things” is a well-known quote. iGEM can never be an exception for this. Whether the work/project is narrowed down or not, one major idea kept in consideration is the “**funding for your project**”.

Few suggestions regarding funding are:

### **You’re first and foremost steps to be followed:**

- If the project idea has been framed, a proposal (brief) should be made, and sent to the sponsors who are to be approached. This is necessary because, the person who is being approached for sponsorship should know the effectiveness of the project.
- Even in the case of not having an idea, you can approach for funding and convince the person regarding your effort by telling the values of iGEM and about your past works (if any).
- You can approach both funding agencies and as well as individual funders whom you think will fund you. Several other places can also be accessed such as industries related to bio-tech, syn-bio or even to your area of research.

- Maintaining a cordial relationship with your sponsors and keeping in touch with them is indeed very important.
- Before you begin with the “sponsor hunt”, you must be clear with your budget and the related documents needed to approach.

Below we have mentioned the names of some Indian as well as international agencies which can positively be accessed for funding and sponsorship:

## **INDIAN AGENCIES:**

Being an Indian team, we know the strategies and also the hardships you may face while approaching for grants. Thus we suggest you to approach the governmental organizations with a properly prepared write-up or proposal. Here we have given a list of governmental and non-governmental organizations in India along with their contact details which can be accessed for funding:

<b>Name of the organization</b>	<b>Contact details</b>
1.Department of scientist and technology.(DST)	+(91)0112301657,26567373,

2.Department of bio-technology(DBT)	011-24363748
3.Department of education(DOed)	011-23782296.
4.Department of food processing industries	011-26492476
5.Department of non-conventional energy sources(DNES)	24361481,24362772.
6.National information system for science & technology.(NISSAT)	011-26567373.
7.Technology absorption and adaption scheme(TAAS)	011-26864570
8.Science and engineering research council(SERC)	011-26963695
9.Science and society related programmes	011-26567373.
10.All Indian Council for Technical Education(AICTE)	91-11-23724183.
11.Council of Scientific and Industrial Research(CSIR)	23710472,23717053.
12.Sri Ratan Tata trust	+91-22-66658282
13.Sri Dorabji Tata trust.	+91 - 22 - 6665 8282
14.Indian development and relief fund	301-704-0032.
15.The Reliance Foundation	091-11-29233392.
16.Narotam Sekhsaria foundation grants	022 61326200

17.Board of research in fusion sciences and technology(BRFST)	07923962062
18.Lady Tata memorial trust	02072358281
19.Intensification of research in high priority areas (DST)	011-26567373
20.Indian National Science Academy (INSA)	23221931

- Apart from the above mentioned agencies and departments, you can very well approach industries and private organizations which are in vicinity to you. Better response may be obtained if you approach an industry related to your project (After framing the idea).
- Note that you should be clear and precise with your work and the possible budget associated with it. There are even organizations that are ready to give travel grants to young researchers. So you can make the best use of it.

## **INTERNATIONAL AGENCIES:**

We have also listed out a set of international funding agencies that can possibly be accessed. This is a general list and not particular to any country or region.



<b>Name of the organisation</b>
1.International foundation for science
2.Third world Academy of sciences
3.Third world network of scientific organizations
4.American academy in Rome
5.Association of commonwealth universities
6.Bradley foundation grant program
7.Ford Foundation
8.The foundation for future
9.Canon foundation fellowships
10.Harry Frank Guggenheim Foundation
11.The Leverhume Trust
12.Indo-French centre for promotion of advanced research. (IFCAPR)
13.UK-India education and research initiative
14.Human Frontier Science Program
15.Japan society for Promotion of research
16.Grantsnet.org
17.The sponsored programs information network (SPIN)
18.The Community of Science (COS)

19. Researchresearch
20. The National Science Foundation (U.S)

- The above list is based on the most frequently sought after trusts and organizations around the world for research based funding.
- However this is not region-specific and may be subjected to changes according to your place of work.

### **FUNDING OPPURTUNITIES PROVIDED BY iGEM:**

iGEM is always there to complement its participants with multitude of opportunities and gateways for their success. In order to accomplish this, it joins hands with many syn-bio based organizations and calls the students for submitting their proposals and make best use of this platform. Those organizations include:

- Twist bioscience and Desktop genetics.
- Genscript
- Syngenta
- Synenergine

Apart from these you can also find a few companies which provide special discounts and offers to support the young and emerging synthetic biologists. The below mentioned set of companies supported the iGEM2016 season.

- Integrated DNA technologies (IDT)
- Genscript
- Autodesk
- Mathworks
- PLOS

## **CROWD FUNDING:**

iGEM also renders crowd funding opportunities to the participants. Crowd funding is a novel concept of pooling in the required fund for a project or venture by raising many small amounts of money from a large number of people, typically via the Internet.

These are the organizations that supported crowd-funding for the iGEMers of the 2016 season

- Indiegogo (global)
  - Walacea.com (global)
  - Experiment.com (US)
  - Idea.me (Latin America)
  - Ulule.com(Europe)
- 
- On the whole, it can be understood that "Funding is provided for those who deserve it".

- Having told that, we would like to insist that a good idea will go places and win several hearts.
- Thus, be precise with your idea, the budget needed for wet lab works and also for other commitments such as travel fare, Registration fee, Accommodation and so on. This will help you to get as much money as possible.

All the best!!